

IN THE SPECIFICATION

The paragraph beginning at page 62, line 23 has been amended as follows:

Q1 To monitor expression of Oct-4, RT-PCR was carried out on colonies consisting predominantly of stem cells, or colonies which had undergone spontaneous differentiation as described below. mRNA was isolated on magnetic beads (DynaL AS, Oslo) following cell lysis according to the manufacturer's instructions, and solid-phase first strand cDNA synthesis was performed using Superscript II reverse transcriptase (Life Technologies). The PCR reaction was carried out according to van Eijk et al. (1999), using the solid phase cDNA as template and Taq polymerase (Pharmacia Biotech, Hong Kong). OCT-4 transcripts were assayed using the following primers: 5'-CGTTCTCTTTGGAAAGGTGTTC (forward) (SEQ ID NO:5) and 3'-ACACTCGGACCACGTCTTTC (reverse) (SEQ ID NO:6). As a control for mRNA quality, beta-actin transcripts were assayed using the same RT-PCR and the following primers: 5'-CGCACCACTGGCATTGTCAT-3' (forward) (SEQ ID NO:7), 5'-TTCTCCTTGATGTCACGCAC-3' (reverse) (SEQ ID NO:8). Products were analysed on a 1.5% agarose gel and visualised by ethidium bromide staining.

The paragraph beginning at page 68, line 2 has been amended as follows:

02 The PCR reaction was carried out according to van Eijk et al. (1999), using the solid phase cDNA as template and Taq polymerase (Pharmacia Biotech, Hong Kong). As a control for mRNA quality, beta-actin transcripts were assayed using the same RT-PCR. PCR primers were synthesized by Besatec or Pacific Oligos (Adelaide, Australia). The following primers were used:

Gene		Primers	Product size
PAX-6	Forward: Reverse:	5'AACAGACACAGCCCTCACAAACA3' (SEQ ID NO:1) 5'CGGGAACCTGAACTGGAACCTGAC3' (SEQ ID NO:2)	274 bp
nestin	Forward: Reverse:	5'CAGCTGGCGCACCTCAAGATG3' (SEQ ID NO:3) 5'AGGGAAGTTGGGCTCAGGACTGG3' (SEQ ID NO:4)	208 bp
Oct-4	Forward: Reverse:	5'-CGTTCTCTTTGGAAAGGTGTTT (SEQ ID NO:5) 3'-ACACTCGGACCACGTCTTTC (SEQ ID NO:6)	320 bp
beta-actin	Forward: Reverse:	5'-CGCACCCTGGCATTGTCAT-3' (SEQ ID NO:7) 5'-TTCTCCTTGATGTCACGCAC-3' (SEQ ID NO:8)	200 bp
CD-34	Forward: Reverse:	5'-TGAAGCCTAGCCTGTCACCT-3' (SEQ ID NO:9) 5'-CGCACAGCTGGAGGTCTTAT-3' (SEQ ID NO:10)	200 bp
FLK-1	Forward: Reverse:	5'-GGTATTGGCAGTTGGAGGAA-3' (SEQ ID NO:11) 5'-ACATTTGCCGCTTGATAAC-3' (SEQ ID NO:12)	199 bp
Hnf-3	Forward: Reverse:	5'-GAGTTTACAGGCTTGTGGCA-3' (SEQ ID NO:13) 5'-GAGGGCAATTCCTGAGGATT-3' (SEQ ID NO:14)	390 bp
AFP	Forward: Reverse:	5'-CCATGTACATGAGCACTGTTG-3' (SEQ ID NO:15) 5'-CTCCAATAACTCCTGCTATCC-3' (SEQ ID NO:16)	340 bp
transferrin	Forward: Reverse:	5'-CTGACCTCACCTGGGACAAT-3' (SEQ ID NO:17) 5'-CCATCAAGGCACAGCAACTC-3' (SEQ ID NO:18)	367 bp

Products were analysed on a 1.5% or a 2% agarose gel and visualized by ethidium bromide staining.

The paragraph beginning at page 71, line 26 has been amended as follows:

03 Differentiation into astrocyte and oligodendrocyte cells was also confirmed at the mRNA level. Spheres were plated on poly-D-lysine and fibronectin and cultured for 2 weeks in the serum free medium supplemented with EGF, bFGF and PDGF-AA. The differentiating spheres were then further cultured two weeks without growth factors and in the presence of T3. RT-PCR was used as above to demonstrate the expression of GFAP and the *plp* gene. GFAP transcripts were assayed using the following primers: 5'-TCATCGCTCAGGAGGTCCTT-3' (forward) (SEQ ID NO:19) and 5'-CTGTTGCCAGAGATGGAGGTT-3' (reverse) (SEQ ID NO:20), band size 383bp. The primers for the analysis of *plp* gene expression were 5'-CCATGCCTTCCAGTATGTCATC-3' (forward) (SEQ ID NO:21) and 5'-GTGGTCCAGGTGTTGAAGTAAATGT-3' (reverse) (SEQ ID NO:22). The *plp* gene encodes the proteolipid protein and its alternatively spliced product DM-20 which are major proteins of brain myelin. The expected band size for *plp* is 354bp and for DM-20 is 249bp (Kukekov et al., 1999). As a control for mRNA quality, beta-actin transcripts were assayed using the same primers as above. Products were analysed on a 2% agarose gel and visualised by ethidium bromide staining.

The paragraph beginning at page 76, line 10 has been amended as follows:

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Colonies of undifferentiated ES cells from the cell lines HES-1 and HES-2 were continuously cultured on mouse embryonic fibroblasts feeder layer for 2-3 weeks. At one week after passage, some spontaneous differentiation was usually identified by changes in cell morphology in the center of the colonies. The process of differentiation included at this early stage the neuroectodermal lineage as evident by the expression of early neural markers such as nestin and PAX-6 (Figure 19). From the second week after passage, areas with differentiated small piled tightly packed cells could be identified in the colonies of both cell lines by phase and inverted microscope. During the third week these areas became more defined from neighboring areas of the colony (Figure 26). The size and demarcation of these areas was enhanced if the serum containing ES cell culture medium was replaced after a week or preferably after two weeks from passage with serum free medium supplemented with EGF (20ng/ml) and FGF (20ng/ml). The cells in these areas were not reactive in immunohistochemical staining with the antibody against the early neuroectodermal marker polysialyated NCAM. The areas were large and well demarcated sufficiently to allow mechanical removal of clumps of cells by a micropipette in 54% of the colonies cultured in serum containing medium (67/124, HES-1). ~~Clumps were~~ Clumps were removed from differentiating colonies of HES-1 and HES-2 and were transferred to serum free medium supplemented with basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). At the time of isolation, the clumps were comprised mostly of a layer of the small tightly packed cells (about 100-300cells/clump), on top of some loosely attached larger cells. It was possible to remove these larger cells mechanically or by enzymatic digestion. Within an hour the clumps started to change their shape toward spheres and after 24 hours all the clumps turned into round spheres (Figure 5a).

IN THE DRAWINGS

Figures 1-7, 9, 11-15, 19-24, and 27-29 have been amended as attached.